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Garcia-Blanco, Mariano A.

<120> METHODS AND COMPOSITIONS FOR USE IN  
SPLICEOSOME MEDIATED RNA TRANS-SPLICING

<130> A31304-B-A 072874.0134

<140> 09/756,095

<141> 2001-01-08

<150> 09/158,863

<151> 1998-09-23

<150> 09/133,717

<151> 1998-08-13

<150> 09/087,233

<151> 1998-05-28

<150> 08/766,354

<151> 1996-12-13

<150> 60/008,317

<151> 1995-12-07

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132

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29

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<213> Corynebacterium diphtheriae

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36

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60  
ttcctgca  
68

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24

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<400> 9  
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25

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16

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<400> 12  
caacgttata ataatgtt  
18

<210> 13  
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<400> 14  
cctggacgcg gaagtt  
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51

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<400> 16  
cttctgtttt ttttctc  
17

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cttctgtatt attctc  
16

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gttctgtcct tgtctc  
16

<210> 19  
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<212> DNA  
<213> Corynebacterium diptheriae

<400> 19  
ggcgctgcag ggcgctgatg atggttggtg  
29

<210> 20  
<211> 36  
<212> DNA  
<213> Corynebacterium diptheriae

<400> 20  
ggcgaagctt ggatccgaca cgatttcctg cacagg  
36

<210> 21  
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<212> DNA  
<213> Corynebacterium diphtheriae

<400> 21  
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21

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atggaatcta cataaccagg  
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<400> 23  
gaaggctgag cactacacgc  
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accggaattc atgaagccag gtacaccagg  
30

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20

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19

<210> 28  
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Escherichia coli lacZ gene

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<220>  
<223> Oligonucleotide primer complimentary to the  
Escherichia coli lacZ gene

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36

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<220>  
<223> Oligonucleotide primers complimentary to the  
Escherichia coli lacZ gene

<400> 30  
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38

<210> 31  
<211> 38  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Oligonucleotide primers complimentary to the  
Escherichia coli lacZ gene

<400> 31  
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38

<210> 32  
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<212> DNA  
<213> Artificial Sequence

<220>  
<223> Oligonucleotide primers complimentary to the  
Escherichia coli lacZ gene

<400> 32  
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<210> 33  
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<212> DNA  
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<220>  
<223> Oligonucleotide primers complimentary to the beta  
HCG6 gene (accession #X00266)

<400> 33  
gcatggatcc tccggagggc ccctgggcac cttccac  
37

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<220>  
<223> Oligonucleotide primers complimentary to the beta  
HCG6 gene (accession #X00266)

<400> 34  
ctgactgcag ggtaaccgga caaggacact gcttcacc  
38

<210> 35  
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<223> Oligonucleotide primers complimentary to the beta  
HCG6 gene (accession #X00266)

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gcatggtaac cctgcagggg ctgctgctgt tgctg  
35

<210> 36  
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<213> Artificial Sequence

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<223> Oligonucleotide primers complimentary to the beta  
HCG6 gene (accession #X00266)

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37

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<220>  
<223> Oligonucleotide primers complimentary to the  
Escherichia coli lacZ gene

<400> 37  
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22

<210> 38

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<220>  
<223> Oligonucleotide primers complimentary to the  
Escherichia coli lacZ gene

<400> 38  
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21

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Escherichia coli lacZ gene (accession #X00266)

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<400> 40  
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45

<210> 41  
<211> 35  
<212> DNA  
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35

<210> 42  
<211> 30  
<212> DNA  
<213> Homo sapiens

<400> 42  
acctctgcag acttcacttc taatgatgat  
30

<210> 43  
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<212> DNA  
<213> Homo sapiens

<400> 43  
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51

<210> 44  
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<400> 44  
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<210> 45  
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<210> 46  
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<212> DNA  
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<400> 46  
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<210> 47  
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<212> DNA  
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<210> 50  
<211> 21  
<212> DNA  
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<400> 50  
cttccttggtta ctccctgtcct g  
21

<210> 51  
<211> 32  
<212> DNA  
<213> Homo sapiens

<400> 51  
gacctctcga gggatttggg gaattatttg ag  
32

<210> 52  
<211> 21  
<212> DNA  
<213> Homo sapiens

<400> 52  
aactagaagg cacagtcgag g  
21

<210> 53  
<211> 24

<212> DNA  
<213> Artificial Sequence

<220>

<223> trans-spliced product containing Human chorionic gonadotropin gene 6 sequences and Corynebacterium diphtheriae diphtheria toxin A sequence

<400> 53  
gagatgttcc agggcgtgat gatg  
24

<210> 54  
<211> 125  
<212> RNA  
<213> Artificial Sequence

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<223> PTM intramolecular base-paired stem

<221> misc\_feature  
<222> (57)...(70)  
<223> Loop comprising a combination of 14 nucleotides according to the specification

<400> 54  
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60  
nnnnnnnnnn aucguuaacu aaauaacuac uaacuaacug ggugaauguu uuuucucggc  
120  
ugcag  
125

<210> 55  
<211> 127  
<212> RNA  
<213> Artificial Sequence

<220>

<223> PTM intramolecular base-paired stem

<221> misc\_feature  
<222> (57)...(70)  
<223> Loop comprising a combination of 14 nucleotides according to the specification

<400> 55  
gcuagccugg gacaaggaca cugcuucacc cgguuaguag accacagccc ugagccnnnn

60  
nnnnnnnnnn aucguuaacu aaauaacuac uaacugggug aacuucugua uuauucugca  
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gcugcag  
127

<210> 56  
<211> 127  
<212> RNA  
<213> Artificial Sequence

<220>  
<223> PTM intramolecular base-paired stem

<221> misc\_feature  
<222> (57)...(70)  
<223> Loop comprising a combination of 14 nucleotides  
according to the specification

<400> 56  
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60  
nnnnnnnnnn aucguuaacu aaauaacuac uaacugggug aaguucuguc cuugucugca  
120  
gcugcag  
127

<210> 57  
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<212> DNA  
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<223> trans-spliced product containing Human chorionic  
gonadotropin gene 6 sequences and Corynebacterium  
diphtheriae diphtheris toxin A sequences

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aaatcttttg tgatggaaaa cttttcttcg taccacggga ctaaactgg ttatgtagat  
120  
tccattcaaa aa  
132

<210> 58  
<211> 18  
<212> DNA

<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived  
from Escherichia coli lacZ gene

<400> 58

gaattcggta ccatgggg

18

<210> 59

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived  
from Escherichia coli lacZ gene and

<400> 59

cgtttacagg taagaggatc ctccggaggg ccc

33

<210> 60

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Artificial sequence comprising sequences derived  
from Escherichia coli lacZ gene

<400> 60

tggtgtcaaa aataataagt taacaagctt

30

<210> 61

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> trans-spliced product containing Escherichia coli  
lacZ gene sequences and Human chorionic  
gonadotropin gene 6 exon 2 sequences

<400> 61

cagcagcccc tgtaaacggg gatac

25

<210> 62

<211> 286

<212> DNA

<213> Artificial Sequence

<220>

<223> trans-spliced product containing Escherichia coli  
lacZ gene sequences

<400> 62

ggcttttcgct acctggagag acgcgcccgc tgatcctttg cgaatacgcc cacgcgatgg  
60  
gtaacagtct tggcgggtttc gctaaatact ggcaggcggt tcgtcagtat ccccgtttac  
120  
agggcggtt cgtctaataa tgggactggg tggatcagtc gctgattaaa tatgatgaaa  
180  
acgggcaacc cgtggtcggc ttacggcggt gattttggcg atacgccgaa cgatcgccag  
240  
ttctgtatga acgggtctggt ctttgccgac cgcacgccgc atccag  
286

<210> 63

<211> 196

<212> DNA

<213> Artificial Sequence

<220>

<223> trans-spliced product containing Escherichia coli  
lacZ gene sequences

<400> 63

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60  
gtaacagtct tggcgggtttc gctaaatact ggcaggcggt tcgtcagtat ccccgtttac  
120  
aggggctgct gctgttgctg ctgctgagca tgggcgggac atgggcatcc aaggagccac  
180  
ttcggccacg gtgccg  
196

<210> 64

<211> 420

<212> DNA

<213> Artificial Sequence

<220>



<223> trans spliced product comprising cystic fibrosis  
transmembrane regulator-derived sequences and His  
tag sequences

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120  
tgatgattat gggagaactg gagccttcag agggtaaaat taagcacagt ggaagaattt  
180  
cattctgttc tcagttttcc tggattatgc ctggcaccat taaagaaaat atcatctttg  
240  
gcggccgcca ctgtgctgga tatctgcaga attccaccac actggactag tggatccgag  
300  
ctcggtagca aggttaagtt taaaccgctg atcagcctcg actgtgcctt ctagttgcca  
360  
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420

<210> 65

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Splice junction sequence

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atgttccagg gcgtgatgat  
20

<210> 66

<211> 7

<212> PRT

<213> Artificial Sequence

<220>

<223> C terminal residues of glutathione-S-transferase

<400> 66

Asp Tyr Lys Asp Asp Asp Lys  
1 5

<210> 67

<211> 15

<212> DNA  
<213> Artificial Sequence

<220>  
<223> Artificial Sequence comprising sequences derived  
from Escherichia coli lacZ gene

<400> 67  
ggagttgatc ccgtc  
15

<210> 68  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Artificial Sequence comprising sequences derived  
from Escherichia coli lacZ gene and human  
chorionic gonadotropin gene 6 intron 1

<400> 68  
gcagtgtcct tgtgcgggta ccctgcaggg cggcttc  
37

<210> 69  
<211> 120  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Binding domain of PTM

<400> 69  
gattcacttg ctccaattat catcctaagc agaagtgtat attcttattt gtaaagattc  
60  
tattaactca tttgattcaa aatattttaa atacttcctg tttcatactc tgctatgcac  
120

<210> 70  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Spacer sequences of PTM

<400> 70  
aacattatta taacgttgct cgaa  
24

<210> 71  
<211> 47  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Branch point, pyrimidine tract and acceptor splice  
site of PTM

<400> 71  
tactaactgg tacctcttct tttttttttg atatcctgca gggcggc  
47

<210> 72  
<211> 70  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Donor site and spacer sequence of PTM

<400> 72  
tgaacggtaa gtgttatcac cgatatgtgt ctaacctgat tcgggccttc gatacgctaa  
60  
gatccaccgg  
70

<210> 73  
<211> 260  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Binding domain of spacer sequence

<400> 73  
tcaaaaagtt ttcacataat ttcttacctc ttcttgaatt catgctttga tgacgcttct  
60  
gtatctatat tcatcattgg aaacaccaat gatttttctt taatgggtgcc tggcataatc  
120  
ctggaaaact gataacacaa tgaaattctt ccactgtgct taaaaaaacc ctcttgaatt  
180  
ctccatttct ccacataatca tcattacaac tgaactctgg aaataaaaacc catcattatt  
240

aactcattat caaatcacgc  
260

<210> 74  
<211> 22  
<212> DNA  
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<220>  
<223> Oligonucleotide primer

<400> 74  
cgctggaaaa acgagcttgt tg  
22

<210> 75  
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<212> DNA  
<213> Oligonucleotide Artificial Sequence

<220>  
<223> Oligonucleotide primer

<400> 75  
actcagtgtg attccacctt ctc  
23

<210> 76  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Oligonucleotide

<400> 76  
gacctctgca gacttcactt ctaatgatga ttatgg  
36

<210> 77  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Oligonucleotide primer

<400> 77

ctaggatccc gttcttttgt tcttcactat taa  
33

<210> 78

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer

<400> 78

ctagggttac cgaagtaaaa ccatacttat tag  
33

<210> 79

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer

<400> 79

gcatggttac cctgcagggg ctgctgctgt tgctg  
35

<210> 80

<211> 37

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer

<400> 80

ctgaaagctt gttaaccagc tcaccatggt ggggcag  
37

<210> 81

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Binding domain of PTM molecule

<400> 81

acccatcatt attaggtcat tat  
23

<210> 82  
<211> 22  
<212> DNA  
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<220>  
<223> Oligonucleotide primer

<400> 82  
gatcaaattct gtcgatacctt cc  
22

<210> 83  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Oligonucleotide primer

<400> 83  
ctgatccacc cagtcccatt a  
21

<210> 84  
<211> 22  
<212> DNA  
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<220>  
<223> Oligonucleotide primer

<400> 84  
gactgatcca cccagtccca ga  
22

<210> 85  
<211> 52  
<212> DNA  
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<220>  
<223> Random sequences inserted to replace 3' splice  
site

<221> misc\_feature  
<222> (7)...(30)  
<223> spacer sequence, see SEQ ID NO:70

<400> 85  
ccgcggnnnn nnnnnnnnnn nnnnnnnnnn gggttccggt accggcggct tc  
52

<210> 86  
<211> 71  
<212> DNA  
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<400> 86  
ttttatcccc gtttacaggg cggttcgctc tgggactggg tggatcagtc gctgattaa  
60  
tatgatgaaa a  
71

<210> 87  
<211> 66  
<212> DNA  
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<220>  
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<400> 87  
tttggcgata cgccgaacga tcgccagttc tgtatgaacg gtctgggtctt tgccgaccgc  
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acgccg  
66

<210> 88  
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<212> DNA  
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<220>  
<223> PTM sequences

<400> 88  
acgagcttgc tcatgatgat catgggcgag ttagaaccaa gtgaaggcaa gatcaaacad  
60

tccggccgca tcagcttttg cagccaattc agttggatca tgcccgggtac catcaaggag  
120  
aacataatct tcggcgtcag ttacgacgag taccgctatc gctcgggtgat taaggcctgt  
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cagttggagg ag  
192

<210> 89  
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<400> 89  
gagcaggcaa gacgagcttg ctcac  
25

<210> 90  
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<400> 90  
gagaacataa tcttcggcgt cagttacg  
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<223> Oligonucleotide

<400> 91  
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30

<210> 92  
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<213> Artificial Sequence



<220>

<223> PTM sequences

<400> 92

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tccggccgca tcagcttttg cagccaattc agttggatca tgcccggtag catcaaggag  
120

aacataatct tcggcgtagc ttacgacgag taccgctatc gctcggtagc taaggcctgt  
180

cagttggagg ag

192

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<211> 27

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide

<400> 93

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27

<210> 94

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide

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30

<210> 95

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> Oligonucleotide

<400> 95

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<210> 96  
<211> 27  
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<223> Oligonucleotide

<400> 96  
aaaatatcat ctttggtggt tcctatg  
27

<210> 97  
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<223> Oligonucleotide

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ccaactagaa gaggacatct ccaagtt  
27

<210> 98  
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<212> DNA  
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<223> 5' splice site

<400> 98  
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21

<210> 99  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> 3' splice site

<400> 99  
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27

<210> 100  
<211> 46  
<212> DNA  
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<220>  
<223> Sequence from trans-splicing domain

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46

<210> 101  
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<211> 323

<212> DNA

<213> Artificial Sequence

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<223> Trans-splicing domain of CFTR PTM

<400> 102

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 120  
 ctgtatctat attcatcatt ggaaacacca atgatatttt ctttaattgg gcctggcata  
 180  
 atcctggaaa actgataaca caatgaaatt ctccactgt gcttaatttt accctctgaa  
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 300

ttaactcatt atcaaatcac gct  
323

<210> 103  
<211> 165  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> PTM binding domain

<400> 103  
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cctaagcaga agtgtatatt cttatttgta aagattctat taactcattt gattcaaaat  
120  
atttaaaata cttcctgttt cacctactct gctatgcacc cgcg  
165

<210> 104  
<211> 225  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Trans-splicing domain of CFTR PTM

<400> 104  
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gcagaagtgt atattcttat ttgtaaagat tctattaact catttgattc aaaatattta  
120  
aaatacttcc tgtttcacct actctgctat gcacccgogg aacattatta taacgttgct  
180  
cgaataactaa ctggtacctc ttcttttttt tttgatatcc tgcag  
225

<210> 105  
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<220>  
<223> CFTR PTM sequence

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agtgggaagaa tttcattctg ttctcagttt tcttggatta tgcttggcac cattaaagaa  
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 aatatcatct ttggtgtttc ctatgatgaa tatagataca gaagcgtcat caaagcatgc  
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 300  
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 360  
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 420  
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